Cardiomyopathy (HCM) patients abolish the relationship between TnI phosphorylation and Ca^{2+}-sensitivity (uncoupling).
Ca^{2+}-sensitisers and Ca^{2+}-desensitisers that act upon troponin alter the Ca^{2+}-sensitivity of the myofilament but their relationship with TnI phosphorylation has never been studied before.
Epigallocatechin-3-gallate (EGCG) is a major extract of green tea and it also acts as a Ca^{2+}-desensitiser by binding to Troponin C of the myofilament. 100 μM EGCG decreased Ca^{2+}-sensitivity of phosphorylated and unphosphorylated wild-type thin filaments equally (by 2.15 ± 0.45 and 2.80 ± 0.48-fold respectively), retaining the coupling. In contrast, EGCG reduced Ca^{2+}-sensitivity of phosphorylated but not unnphosphorylated thin filaments containing 8 DCM (*TPM1* E54K and E40K, *TNNC1* G159D, *TNNI3* K36Q, *ACTC* E361G) and HCM (*TPM1* E180G, *TNNT2* K280N, *ACTC* E99K)-causing mutations. As a result the dependence of Ca^{2+}-sensitivity upon TnI phosphorylation of uncoupled mutant thin filaments was restored in every case. In single myofibrils, EGCG reduced Ca^{2+}-sensitivity of force and k_{ACT} and also preserved the coupling with wild type and restored coupling with *ACTC* E361G mutant myofibrils.
The effect of EGCG demonstrates that it is possible to reverse the pathological defects in troponin caused by HCM mutations pharmacologically.

1817-Plat
Myosin-Binding Protein C Corrects an Intrinsic Non-Uniformity in Cardiac Excitation-Contraction Coupling
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Cardiac contraction is initiated by calcium release from the sarcoplasmic reticulum through channels (ryanodine receptors) that are located near the sarcomere ends. Once released, calcium must diffuse towards the sarcomere center to fully activate the actomyosin contractile system. This physical arrangement should lead to a spatial calcium gradient and thus non-uniform contractile activation. We hypothesize that myosin-binding protein C (MyBP-C), a potent thin filament activator, is localized to the sarcomere center (C-zone) to mitigate the potential deficit in calcium activation. We used EM and super-resolution STORM microscopy to visualize the relative positions of the ryanodine receptors and MyBP-C within the sarcomere. Laser scanning confocal microscopy of calcium transients in isolated cardiac cells showed that calcium concentrations at the center of each sarcomere lagged those near the ends of each sarcomere by as much as 150 nM. The functional impact of this calcium gradient was determined by examining the sliding of native, calcium-sensitive actin-thin filament shards over native mouse cardiac myosin-thick filaments using a TIRFM-based motility assay. The presence of MyBP-C enhanced the fraction of thin filaments moving within the thick filament C-zone (pCa50 6.5 ± 0.04 vs 6.4 ± 0.02; $p<0.01$). 3D EM reconstructions of native thin filaments suggest this calcium sensitization results from MyBP-C's N-terminal domains shifting tropomyosin on the thin filament. Using an analytic model, we show that MyBP-C residing within the C-zone can counterbalance differences in calcium activation within the sarcomere during the early phase of contraction. Thus, MyBP-C's localization to the C-zone may function to correct an intrinsic defect in cardiac excitation-contraction coupling, and any disturbance of MyBP-C localization or function will contribute to the consequent cardiac pathologies.

1818-Plat
Direct Detection of the Thermodynamics and Structural Kinetics of a 2-Color SERCA Biosensor by Transient Time-Resolved FRET
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We are investigating the structural dynamics of the calcium pump SERCA, using a 2-color biosensor and time-resolved FRET measurements. SERCA couples ATP hydrolysis to Ca^{2+} transport across the sarcoplasmic or endoplasmic reticulum membrane. Crystal structures suggest that SERCA structure changes dramatically during active Ca^{2+} transport, including large movements in the cytoplasmic N and A domains, coupled to more subtle changes in the transmembrane domain. However, there is little direct evidence showing how changes in SERCA structure coordinate, in solution and in cells, with individual steps in the pump's ATPase and Ca^{2+} transport cycles. Furthermore, SERCA is a viable therapeutic target for a host of diseases_heart failure, diabetes, and cancer_so it is important to understand how the ATPase and Ca^{2+} transport cycles are coupled to changes in the protein's structure. We are addressing these questions using a recombinant 2-color SERCA biosensor (RFP and GFP fused to the N and A domains) developed by Robia and coworkers for live-cell work. Here we use it in isolated membrane fragments to detect changes in SERCA structure in response to changes in Ca^{2+} and nucleotide concentrations, both at equilibrium and in the transient phase after rapid mixing. Our results: 1) reveal critical changes in the structural-dynamics of the SERCA cytoplasmic domains upon Ca^{2+} and nucleotide binding; and 2) suggest a structural-kinetic mechanism for Ca^{2+} activation of ATPase cycling with coordinated changes in the position of the cytoplasmic domains in association with sequential binding of Ca^{2+} ions and ATP. 3) These results also allow direct testing of x-ray crystal structure models in solution and in living cells and inform the discovery of novel SERCA modulators that directly target the pump's structural dynamics.

1819-Plat
Phospholamban-Independent Adrenergic Reserve in SERCA2 Ablated Hearts
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Normal cardiac contraction and relaxation requires efficient operation of the SR Ca^{2+} ATPase, SERCA2a. In failing hearts, decreased SERCA2a expression and slowed SR Ca^{2+} reuptake are thought to significantly underlie contractile dysfunction. We have studied the relationship between decreased SERCA2a expression and cardiac function using a mouse model of conditional cardiac Serca2 ablation, the Serca2fl/fl mouse. In this model, Cre activation by tamoxifen injection efficiently deletes Serca2 from the heart; four weeks post-knockout, cardiac SERCA2a protein content is below 5% of normal levels. Serca2 KO mice survive 7-10 weeks post knockout with only mild in vivo impairment prior to this time, suggesting that this loss of SERCA2a protein can be temporarily compensated. We found that isolated SERCA2a KO hearts retained the ability to respond to ß-adrenergic stimulation ex vivo despite the loss of most SERCA2a protein. Because the protein regulator of SERCA2a, phospholamban (PLN), is thought to be a major component of the cardiac ß-adrenergic response, this finding was unexpected and warranted further detailed investigation. To identify the mechanism underlying this preserved ß-adrenergic response in SERCA2-depleted hearts, we bred Serca2fl/fl and PLN-/- mice to generate an inducible Serca2 knockout mouse line lacking PLN. Serca2fl/fl;PLN-/- mice were injected with tamoxifen to induce Serca2 gene disruption, and isolated hearts from Serca2KO;PLN-/- (''DKO'') mice were evaluated by Langendorff perfusion 4-5 weeks after Serca2 knockout. Control PLN-/- hearts show little response to ß-adrenergic stimulation, and DKO hearts have impaired contractility under baseline conditions. DKO hearts, however, show improved systolic and diastolic function when perfused with 50 nM isoproterenol, indicating that targets of ß-adrenergic signaling beyond PLN are able to support a significant inotropic and lusitropic response when cardiac contractility is impaired. We will discuss how these findings support new mechanisms underlying heart performance during stress and disease.

Platform: Protein Dynamics and Allostery II

1820-Plat
Conformational Dynamics of Single HIV-1 Envelope Proteins on the Surface of Native Virions
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The HIV-1 envelope (Env) mediates viral entry into host cells. While static images of Env in unliganded and ligand-bound forms define distinct